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## ABSTRACT

The effects of homogenization of cheese milk and proteolysis in cheese on formation and thermal properties of free oil in Mozzarella cheese were examined. The amount of free oil generated by heating Mozzarella depended on interactions between casein and fat. Homogenization of cheese milk greatly reduced free oil; homogenization of skim milk alone had no effect. When the milk was not homogenized, free oil formation increased with the percentage of fat in DM and protein breakdown. Thermal profiles of cheese fat, free oil, and milk fat were similar, although cheese fat melted at a higher temperature, and free oil from homogenized Mozzarella melted at a lower temperature. Heat of fusion and fatty acid composition of free oil were not different from those of cheese fat. (**Key words**: free oil, homogenization, Mozzarella cheese, proteolysis)


**Abbreviation key**: **DSC** = differential scanning calorimetry, **FDM** = fat in DM, **FO** = free oil, **FOFB** = FO on a fat basis, **MP** = melting point.

## INTRODUCTION

Mozzarella cheese tends to form free oil (**FO**) upon melting, but excessive amounts of FO give the cheese an undesirable appearance. This phenomenon, which is also known as oiling off or fat leakage, occurs when the casein matrix collapses during heating, allowing the fat globules to coalesce and to flow to the surface. The FO on a fat basis (**FOFB**) is defined as percentage of FO divided by percentage of total fat; in Mozzarella cheese, FOFB is dependent on fat in DM (**FDM**) when the value of the latter is higher than about 37% (11). Above this critical percentage, the surface area of the fat is too great for complete emulsification by casein (19). Refrigerated storage of Mozzarella increases the amount of FO (11). This effect is presumably due to proteolysis (11), although a detailed study has not been reported. Homogenization of milk prior to Mozzarella cheese manufacture reduces the average size of the fat droplets, which greatly increases the degree of emulsification (13). As a result, virtually no FO forms when homogenized cheese is heated (3, 6, 7, 13). The changes that milk proteins undergo during homogenization could affect emulsification. This possibility can be investigated by homogenizing skim milk and then adding cream prior to cheese making.

Differential scanning calorimetry (**DSC**) is well suited for investigating the thermal properties of FO and cheese fat, although no studies have been reported. Milk fat undergoes a melting transition, which is easily observed by DSC, from about –30 to 38°C; casein does not exhibit thermal transitions in this temperature region. Therefore, fat is the only visible component in a DSC curve of cheese if the sample is dried prior to analysis. The resulting thermal profile provides the melting point (**MP**) and heat of fusion of the fat that is contained in the sample.

Differences between the fatty acid profiles of FO and non-FO fat could also have an effect on FO formation and melting, but no such comparisons have been reported. Formation of FO could be reduced by addition of emulsifying salts or increased pH of the cheese (19), but the former is not permitted according to the Federal Standards of Identity for Moz-


Received July 26, 1993.
Accepted April 25, 1994.


[1]Mention of brand name or firm name does not constitute endorsement by the USDA over others of a similar nature not mentioned.

zarella (5), and the latter adversely affects the stretchability of the product (12). The objective of this research was to compare the thermal properties of FO and non-FO fat from Mozzarella cheese and to determine how FO formation is affected by homogenization of casein, proteolysis, and fatty acid composition.

## MATERIALS AND METHODS

### Cheese Preparation

Full fat, low moisture Mozzarella cheeses were made from three types of milk: nonhomogenized, homogenized, and homogenized skim to which nonhomogenized cream was added. Milk (45.4 kg) was divided into two portions; one batch of cheese was prepared from each half on consecutive days. Milk intended for nonhomogenized and homogenized cheeses was standardized with cream or skim milk to 3.5% fat content; the fat was removed from the milk intended for homogenized skim cheese. All milk was then pasteurized at 63°C for 30 min. The milk for homogenized and homogenized skim cheeses next underwent two-stage homogenization at 63°C at pressures totaling 10,300 kPa (25). The unhomogenized cream was then added back to its homogenized skim milk until the fat content was 3.5%.

Mozzarella cheese was prepared according to the procedures previously described (23, 24, 25). Cheese milk at 32.4°C was inoculated by direct addition of 125 ml of CR7 starter culture (Marschall Rhône-Poulenc, Madison, WI), which was described by the manufacturer as consisting of 50% *Streptococcus salivarius* ssp. *thermophilus* and 50% *Lactobacillus delbruekii* ssp. *bulgaricus*. After the pH decreased .1 unit, 4.4 g of number 01034 single-strength calf rennet (Chr. Hansen's Laboratory, Milwaukee, WI) were added. The curd was held for 35 min, cut, held for another 15 min, stirred at 32.4°C for 10 min, and held unstirred at that temperature for 90 min. The whey (pH 6.3 to 6.4) was then drained, and the curd was rinsed with tap water at 25°C, cut into slabs, and cheddared. When the pH decreased to 5.2 to 5.3, the slabs were covered and iced overnight. The next day, the curd was divided into eight parts and stretched and kneaded multidirectionally by hand for 7 min in water at 70 to 80°C. The samples were pressed into 224-ml polyethylene cups (approximately 80 mm in diameter and 55 mm high), cooled, removed from the cups, brined for 2 h in 23% salt solution at 25°C, blotted dry with clean paper towels, and stored in vacuum-sealed pouches at 4°C for up to 6 wk. Two cheese types were prepared each week over a period of several months in a completely random design, with three replicates of each type. Each sample was analyzed after 1 and 6 wk of storage.

### Determinations of Moisture, Fat, and Salt

Compositional analyses included determinations of moisture by the forced-draft oven method (1) at 130°C for 75 min, fat by the modified Babcock test (12), and salt by chloride ion electrode (Orion Research, Inc., Boston, MA).

### Determination of FO

The procedure of Kindstedt and Rippe (11) was used to measure FO in duplicate. A wedge of cheese extending from the surface to the center was removed by knife and ground for several seconds in a blender until particles were <5 mm. Eighteen grams were weighed into a 50% Paley-Babcock bottle; a 20% bottle was used for samples made from homogenized milk. The bottle was immersed in boiling water for 4.0 min, and 20 ml of hot (57.5°C) distilled water were immediately added. The bottle was centrifuged in a Babcock centrifuge at about 57.5°C for 10 min, and 50% methanol was added until the top of the liquid was high in the calibrated neck. The bottle was centrifuged at about 57.5°C for 2 min and rocked gently by hand for 10 s to dislodge air bubbles and fat droplets. The 2-min centrifugation was repeated twice with 10-s intervals of rocking. The bottle was held in the centrifuge for 5 min before the height of the fat column to the bottom of the meniscus was measured. Glymol was not used to eliminate the meniscus. The height of the fat column was divided by 2 and then by the percentage of total fat in the sample to obtain the percentage of FOFB. An aliquot of the FO was taken by pipet for DSC and GLC studies. In samples without a measurable fat column, the oil droplets visible at the meniscus were pipetted along with methanol-water solution into a DSC sample pan. The sample was dried in an oven at 105°C for several minutes, and the weight of remaining fat was used to calculate FOFB.

#### Electrophoresis

Cheese samples were grated for protein analysis, and 2 g were added to 5 ml of buffer solution consisting of .166 *M* Tris (Sigma Chemical Co., St. Louis, MO) and 1 m*M* EDTA (Fisher Scientific, Pittsburgh, PA). The extractions were conducted in a 30-ml cup of a Virtis model S23 homogenizer (Virtis Co., Gardiner, NY). The sample was mixed at 70% of full speed for 15 min, 5 ml of 7% SDS (Sigma Chemical Co.) in buffer were added, the sample was mixed at 50% of full speed for 5 min, 2 ml of 10 m*M* dithiothreitol (Calbiochem, San Diego, CA) in buffer were added, and the sample was mixed well and held in an ice bath for 20 to 30 min. Samples were then centrifuged 1 h at 4°C at 18,000 rpm in an SS34 rotor of a Sorvall RC5C centrifuge (DuPont Co., Newtown, CT). The supernate was filtered through laboratory wipe tissues and lyophilized. The resulting extracts were stored at –20°C. The SDS-PAGE of extracts was performed with the PhastSystem® (Pharmacia LKB Biotechnology, Piscataway, NJ) using 20% homogeneous gels. Gels were stained with a .1% solution of Coomassie blue R250 (Pharmacia LKB Biotechnology), destained, and dried. A Bio-Rad model 620 Video Densitometer (Bio-Rad, Richmond, CA) interfaced with a computer, and 1D Analyst II (Version 3.10) software (Bio-Rad) was used to scan the gels and to integrate peak areas.

#### DSC

Thermal characteristics were measured with a Perkin-Elmer DSC-7 (Perkin-Elmer Corp., Norwalk, CT), equipped with a Perkin-Elmer Intracooler II refrigeration unit. The purge gas was $N_2$ at 20 ml/min. The instrument was calibrated with high purity indium. About 20 to 30 mg of cheese were placed into an Al DSC sample pan (Perkin-Elmer) and then dried in a forced-draft oven at 130°C for 20 min. The dry weight was then multiplied by the percentage of fat in the cheese to obtain the weight of fat in the pan. Samples of FO (up to 12 mg) were pipetted directly into the pan and weighed. An empty sample pan was used as a reference. Samples were tempered in the instrument by holding at 60°C for 5 min, cooling to –50°C at 5°C/min, and holding at –50°C for 5 min. A DSC curve was then obtained by heating the sample to 50°C at 5°C/min. A blank curve, obtained by tempering and heating two empty pans, was then subtracted from the sample curve using the DSC data analysis program. The temperature at which melting of fat was complete was designated the MP. The heat of fusion, in joules per gram of fat, was determined by dividing the area beneath the curve by grams of fat in the pan.

#### GLC

Cheese samples were grated by hand and extracted three times with diethyl ether (J. T. Baker Inc., Phillipsburg, NJ) to obtain cheese fat. Internal standards (standard mix M-100; Nu-Chek-Prep, Elysian, MN) and .5*N* NaOH were added to fat and FO samples, which were heated in a boiling water bath for 15 min (20). Samples were converted into methyl esters with $BF_3$-$CH_3OH$ reagent, and acyl composition was determined by GLC using a Hewlett-Packard model 5895 chromatograph (Avondale, PA) equipped with a split capillary injector, flame-ionization detector, and a Hewlett-Packard model 3396 integrator (4). Separations were obtained on a .25 mm i.d. × 30 m SP-2340 column (Supelco Inc., Bellefonte, PA). The carrier gas was helium with linear velocity of 22.9 cm/s at an 80:1 split ratio. Elution of methyl esters was carried out with temperature programming from 140 to 155°C at .5°C/min and 155 to 200°C at 2°C/min.

#### Statistical Analysis

The responses FOFB, MP of FO, MP of cheese fat, heat of fusion of FO, heat of fusion of cheese fat, and $\alpha_{s1}$-casein were analyzed by the general linear models procedure of SAS (17) for the effects of cheese type and storage time. An effect or interaction was described as significant when $P < .05$.

## RESULTS AND DISCUSSION

Percentages of fat, moisture, and salt in the three types of cheese are shown in Table 1. The ANOVA are given at Table 2.

TABLE 1. Fat in DM (FDM), moisture, and salt in Mozzarella cheeses.

| Milk used | FDM | Moisture | NaCl |
|---|---|---|---|
| | | (%) | |
| Nonhomogenized | 47.2[a] | 47.8[a] | 2.6[ab] |
| Homogenized skim | 50.4[b] | 45.7[a] | 2.5[a] |
| Homogenized | 50.5[b] | 48.4[a] | 3.0[b] |
| SE | .4 | 1.2 | .07 |

[a,b]Means within column with like superscripts do not differ ($P < .05$).

Cheeses made from homogenized milk exhibited low FOFB; the FOFB in the nonhomogenized cheeses was higher and was not significantly different from the FOFB in the homogenized skim cheeses (Table 3). These findings indicate that a reduction in the size of the fat droplets, but not homogenization of protein, was a key to reduction of FO. The FDM in the homogenized skim and nonhomogenized cheeses was high enough to prevent complete emulsification of fat (10, 11). The interaction of type × week for FOFB (Table 2) was evidenced by the significant effect of storage time in the nonhomogenized and homogenized skim cheeses and by the insignificant effect in the homogenized cheeses. The comparatively high salt concentration in the cheeses probably also decreased FO formation.

The relatively low cooking temperature of 32.4°C was selected to determine whether proteolysis of casein significantly affects FO formation, as others (9, 11) have surmised. In Mozzarella cheeses cooked at a reduced temperature, proteolysis of $\alpha_{s1}$-casein increased because the rennet and starter bacteria were probably not inactivated (9, 23, 24, 25). Table 3 shows that FO increased significantly, and $\alpha_{s1}$-casein decreased significantly, during storage of the cheeses.

The DSC curves of a typical nonhomogenized Mozzarella and its recovered FO are shown in Figure 1; the corresponding curves from homogenized skim cheese are identical. Figure 2 shows the DSC curves of residual fat and FO in homogenized cheese; the general shapes of these curves are similar to DSC curves of milk fat obtained by others (14, 18, 22).

The MP of the FO from the nonhomogenized and homogenized skim cheeses were not significantly different but were significantly higher than the MP of the FO from the homogenized cheeses (Table 3). The smaller droplets in the homogenized fat appar-

TABLE 2. The ANOVA for effects of various factors on different parameters of Mozzarella cheese.

| | Source | | | |
|---|---|---|---|---|
| | Type | Week | Type × week[1] | Residual |
| df | 2 | 1 | 2 | 12 |
| | | MS[4] | | |
| FOFB[2] | 4187*** | 610*** | 93.4** | 10.7 |
| MP of FO[3] | 8.28** | .125 | .315 | .741 |
| MP of non-FO fat in cheese | 5.39 | .094 | .467 | 2.34 |
| Heat of fusion | | | | |
| FO | .647 | .005 | 2.91 | 2.62 |
| Non-FO in cheese | 4.13 | 47.7 | 14.4 | 11.0 |
| $\alpha_{s1}$-Casein | 23.0 | 485* | .307 | 56.5 |

[1]Effects and interactions of cheese type (type) and weeks of storage (week) tested against residual error.

[2]Free oil on a fat basis.

[3]Melting point of FO.

[4]Type I mean squares.

*$P < .05$.

**$P < .01$.

***$P < .001$.

TABLE 3. Mean values of free oil (FO) on a fat basis (FOFB), melting point (MP), heat of fusion, and $\alpha_{s1}$-casein in Mozzarella cheeses.

| | Non-homogenized | Homogenized skim | Homogenized | $\overline{X}$ | SE |
|---|---|---|---|---|---|
| FOFB, % | | | | | |
| 1 wk | 34.4$^{d}$ | 43.3$^{c}$ | .6$^{e}$ | | 1.9 |
| 6 wk | 50.8$^{b}$ | 59.3$^{a}$ | 3.1$^{e}$ | | |
| MP of FO, °C | | | | | |
| 1 wk | 36.9 | 36.2 | 34.9 | | |
| 6 wk | 37.1 | 36.8 | 34.6 | | |
| $\overline{X}$ | 37.0$^{a}$ | 36.5$^{a}$ | 34.8$^{b}$ | | .35 |
| MP of non-FO fat in cheese, °C | | | | | |
| 1 wk | 38.5 | 39.0 | 40.1 | | .62[1] |
| 6 wk | 38.9 | 38.5 | 40.6 | | |
| Heat of fusion, J/g of fat | | | | | |
| FO | | | | | |
| 1 wk | 78.0 | 79.4 | 78.7 | | .66[1] |
| 6 wk | 79.6 | 78.5 | 78.0 | | |
| Non-FO in cheese | | | | | |
| 1 wk | 80.1 | 80.0 | 80.1 | | 1.4[1] |
| 6 wk | 78.1 | 79.0 | 77.3 | | |
| $\alpha$-Casein, % | | | | | |
| 1 wk | 41.7 | 44.7 | 44.7 | 43.7$^{a}$ | 3.1 |
| 6 wk | 30.8 | 34.6 | 30.8 | 32.1$^{b}$ | |

$^{a,b,c,d,e}$Means within category with different superscripts differ ($P < .05$).

[1]None of the parameters in this category were different ($P > .05$).

ently complete their melting faster than the larger droplets in nonhomogenized milk. Increasing the FDM had no effect on the MP of the FO in the nonhomogenized and homogenized skim cheeses but tended to increase the MP of the homogenized cheese FO. The MP of the fat in the three types of cheese were not significantly different but were significantly higher than the MP of the corresponding FO. The enclosure of the fat glo-

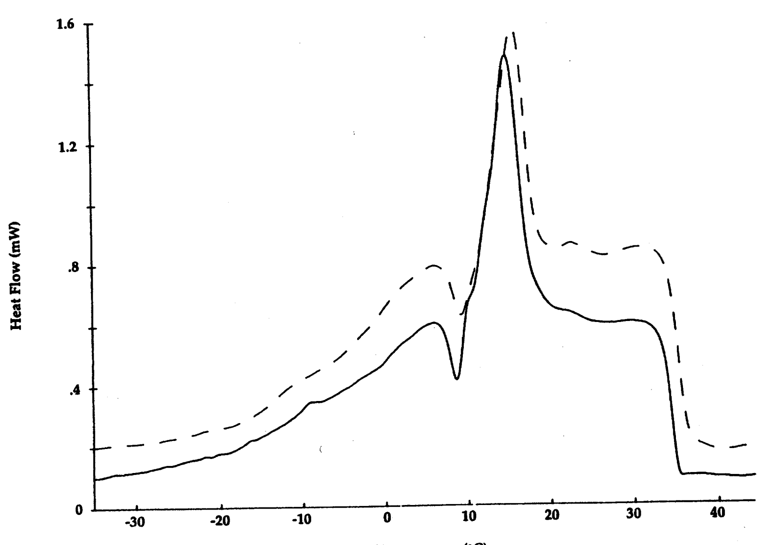


Figure 1. Melting profiles of free oil (FO) and non-FO fat from a Mozzarella cheese sample prepared from homogenized milk: 3.72 mg of FO (—); 9.32 mg of dried cheese (- - -).

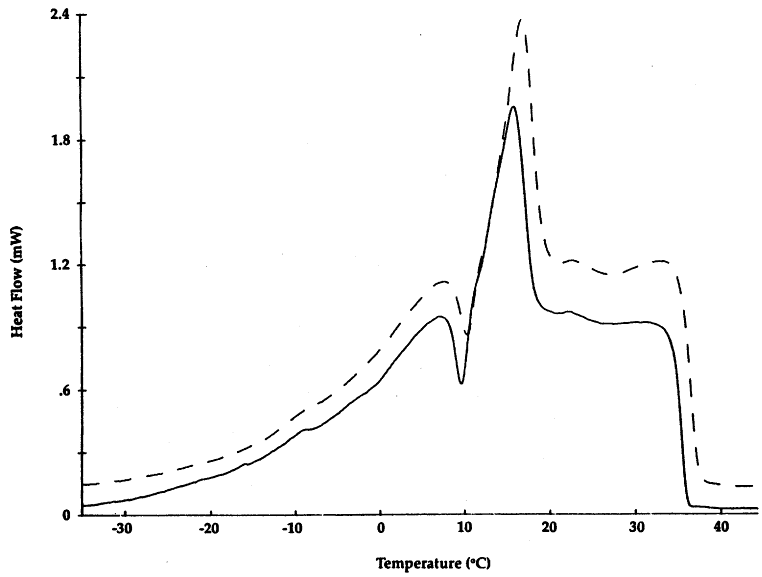


Figure 2. Melting profiles of free oil (FO) and non-FO fat from a Mozzarella cheese sample prepared from nonhomogenized milk: 6.06 mg of FO (—); 13.80 mg of dried cheese (- - -).

bules by protein is evidently responsible for this late melting. Storage time did not affect the MP of cheese fat or FO. Various researchers (2, 15, 16, 21) have analyzed bovine milk fat by DSC and have consistently determined that melting is complete between 36 and 37°C, which is in agreement with our data for the FO from the nonhomogenized and homogenized skim cheeses.

The heats of fusion of the FO and cheeses were not significantly different and exhibited no significant trends (Table 3). Reported or calculated literature values (2) for heat of fusion of milk fat range from 60 to 95 J/g. Differences in DSC procedures, particularly tempering, probably account for the variation (2).

TABLE 4. Percentages[1] of major fatty acids in free oil (FO) and non-FO fat in Mozzarella cheese.

| Fatty acid[2] | FO | Non-FO Fat | SE |
|---|---|---|---|
| | | (%) | |
| 4:0 to 10:0 | 5.9 | 5.7 | .5 |
| 12:0 | 3.2 | 3.0 | .1 |
| 14:0 | 10.4 | 10.4 | .1 |
| 16:0 | 29.8 | 30.1 | .2 |
| 18:0 | 10.9 | 10.6 | .3 |
| 18:1 | 26.2 | 26.8 | .4 |
| 18:2 | 3.3 | 3.5 | .03 |

[1]Mean of three replicates.

[2]Number of carbons:double bonds.

To determine whether the residual fat in the cheese contained fatty acids that were higher melting and less mobile than those in the FO, the fatty acid profiles of the FO samples were compared with those of the non-FO fat extracted from the cheeses. Table 4 shows that the fatty acid profiles of the FO and cheese fat were not significantly different, which indicates that lower melting fatty acids do not migrate to the FO. In addition, the profiles were similar to typical profiles for milk fat (8). Thus, fatty acid composition did not play a role in formation or thermal properties of FO.

## CONCLUSIONS

Homogenization of milk virtually eliminated FO formation in Mozzarella cheese, although homogenization of casein had no effect. When fat was not homogenized, FO formation was dependent on proteolysis. The MP of nonhomogenized FO was about 37°C, which was the same as that of milk fat; the MP of homogenized FO was about 2°C lower. The MP of non-FO fat in Mozzarella was elevated, presumably because casein surrounds the fat droplets. The heat of fusion of the FO and the fat in the cheese was around 79 J/g. The FO and non-FO fat had similar fatty acid compositions.

## ACKNOWLEDGMENTS

The author thanks Philip W. Smith for cheese preparation, Thomas A. Foglia and John G. Phillips for fatty acid and statistical analyses, respectively, James J. Shieh and Brien C. Sullivan for electrophoretic analyses, Edyth L. Malin for interpretation of the proteolysis data, and Andre Rubin for technical assistance.

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